

REMARKS

The Office Action has been carefully reviewed. Claims 17-21 are allowed. Claims 1-7, 9-16 and 28-30 also appear in this application and define patentable subject matter warranting their allowance. Reconsideration and allowance are hereby respectfully solicited.

Claims 1-5, 10-16 and 28-30 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. This rejection is respectfully traversed.

In the examiner's response to applicant's arguments, the examiner states that the 1.132 Declaration by Keith Foster (it appears that the examiner meant to refer instead to the declaration of David Karaolis, as there is no declaration of Keith Foster filed in this application) has been fully considered but was not deemed persuasive. The examiner further states that:

(i) the *Staphylococcal aureus* experimental results in the present specification do not provide enablement for a gram positive bacteria;

(ii) the claims encompass any bacterial pathogen which includes any type of bacteria;

(iii) the instant claims are drawn to reducing the virulence of a bacterial pathogen and not limited to the reduction in colonization, concluding that the experimental data

as disclosed in the declaration are not commensurate in scope with the claims;

(iv) the limited number of species disclosed is not deemed to be representative of the genus encompassed by the claims;

(v) declarant's argument that cyclic dinucleotides would have been a matter of routine is not germane because of applicant's disclosure that specific cyclic dinucleotides may act as either agonists or antagonists of c-di-GMP, which applicant states is a property that can be rapidly and readily determined with only routine experimentation using biofilm formation/inhibition assays in microtiter plates, test tubes or flasks, as disclosed in paragraph [0045] and in the examples of the specification; and

(vi) the claims are drawn to any cyclic dinucleotide and therefore using any common structure of a cyclic dinucleotide in the methods as claimed is unpredictable based on the teachings of Bowie et al., *Science* 247:1306-1310 (1990).

Regarding points (i), (ii) and (iv), which are related, applicant previously presented and discussed in his declarations filed October 6, 2008 and September 15, 2009, the experimental results from the manuscripts, abstracts and publications that were attached to the declarations, as summarized in the Summary Table attached hereto. The examiner's attention is respectfully

invited to not only the disclosure in the present specification (ref. 1 in the Summary Table) or the disclosures of the Karaolis et al. (ref. 2 in the Summary Table), where *S. aureus* colonization and infection in mice were inhibited by c-di-GMP, but also to the Ogunniyi et al. manuscript (ref. 4), where colonization with *Streptococcus pneumoniae*, a type of gram-positive bacterial pathogen different from *Staphylococcus aureus*, and with *Pseudomonas aeruginosa*, a further type of gram-positive bacterial pathogen, are reduced or inhibited by c-di-GMP. Insofar as gram-negative bacterial pathogens are concerned, the attached Summary Table summarizes experimental results in four different gram negative bacteria pathogens, *Klebsiella pneumoniae*, *Brucella melitensis*, *Ehrlichia chaffeensis* (which is similar to *Rickettsia*) and *Vibrio parahaemolyticus*, showing that colonization and/or virulence were reduced/inhibited.

As applicant has provided experimental support in a total of seven distinct species of bacterial pathogens that span a wide spectrum (since they are all quite different and unrelated) within the genus of bacterial pathogens and nearly evenly divided among gram-negative and gram-positive bacterial pathogens, those of skill in the art would most certainly consider such a number of species spanning a broad spectrum to be representative of the genus of bacterial pathogens. There is no requirement that the colonization and virulence of every

bacterial pathogen be inhibited or reduced, but rather that one of skill in the art following the guidance set forth in the present specification can reasonably expect and readily determine with only routine experimentation that colonization and virulence of most other bacterial pathogens, as predicted by the applicant (see the numerous, but non-limiting, examples of bacterial pathogens disclosed in paragraph [0049] of on pages 23-25 of the present specification), would be inhibited or reduced.

The preferred cyclic dinucleotide for use in the presently claimed method is cyclic-di-GMP, whose structure is shown in paragraph [0014] of the specification and described as two GMP molecules bound in a head-to-tail arrangement. All nineteen representative examples of cyclic dinucleotides, compounds (I)-(XIX), shown on pages 19-21 of the present specification are in this arrangement of two nucleotides bound head-to-tail, and this head-to-tail arrangement is what was intended by the applicant when referring to cyclic dinucleotides. As far as applicant is aware, there is no other arrangement of cyclizing two nucleotides except in a head-to-tail arrangement. Applicant proposes to amend claim 1 to recite in the interest of clarity that the cyclic dinucleotides have their nucleotides in a head-to-tail arrangement, even though such an amendment is not needed for a full understanding of what is encompassed by cyclic dinucleotides to those of skill in the art, especially in view of

the disclosure of cyclic dinucleotides in the present specification.

Regarding point (vi), contrary to the complexities of protein primary, secondary and tertiary structure and the problem of predicting protein structure from sequence data, applicant re-emphasizes that a simple small molecule of two nucleotides, cyclized into an invariant head-to-tail arrangement of the ribose sugar moieties of the nucleotides bound together by monophosphates is not analogous to protein structure. This head-to-tail arrangement of the ribose sugar moieties of the nucleotides bound together by monophosphates is a "core structure" that is invariant. As shown in the non-limiting examples of cyclic dinucleotides on pages 19-21 of the specification and the teaching at the top of page 22, the guanine base of each nucleotide in c-di-GMP can be substituted with other bases. While the base (e.g., guanine, adenine, etc.) is variable, as well as some substituents of the ribose moieties, the overall structure of the cyclic dinucleotides is mostly the same based on the common core structure. Accordingly, the unpredictability associated with protein structure as taught by the cited Bowie et al. reference is irrelevant to cyclic dinucleotides, which can be expressed by a common structural formula where variability resides in only the bases and in the

substituent groups of the common core structure of two ribose moieties bound in a head-to-tail arrangement by monophosphates.

Turning to point (v), the present specification teaches that one of skill in the art can readily determine the effect of c-di-GMP or another cyclic dinucleotide on the biofilm formation of any bacterial pathogen by conducting quick and easy assays in microtiter plates, as exemplified in Example 3, paragraph [00101], pages 53-54. One of skill in the art would only use routine experimentation to conduct the assay by incubating the bacterial pathogen with a cyclic dinucleotide in microtiter plates, then washing and staining the wells to reveal whether or not biofilm formation by the bacterial pathogen was inhibited. As would be instantly recognized by those of skill in the art, the use of microtiter plates allows the screening of a multitude of different bacterial pathogens and/or a multitude of different cyclic dinucleotides in microtiter plates with a rapid turnaround period of just a few days. Given the guidance provided by the present specification, those of skill in the art would be well enabled to quickly and easily determine, with minimal experimentation that can only be considered routine, the cyclic dinucleotides that would inhibit biofilm formation of biofilm forming bacterial pathogens.

As for point (iii), those of skill in the art (i.e., in medical/pathogenic bacteriology) would fully understand the

interrelationship between the terms "infection", "pathogenicity", "virulence" and "colonization". It is clear to those of skill in the art, as evidenced by the attached pertinent pages of the textbook Thomas Brock, BIOLOGY OF MICROORGANISMS, 3rd edition, Prentice-Hall, 1979, that virulence is the relative ability of a pathogen to cause disease/infection, otherwise known as its degree of pathogenicity. Colonization, such as by biofilm formation, is the first step in the infection process and is part of the virulence of a bacterial pathogen (see the attached pages from the Dr. Kenneth Todar's Online Textbook of Bacteriology, 2008, at the website www.textbookofbacteriology.net). When a bacterial pathogen is able to readily colonize and invade a host, it is said to be highly virulent. Accordingly, the experimental data in Example 8, page 80, of the present specification demonstrating that c-di-GMP inhibits the ability of *S. aureus* to colonize and infect the mammary gland of mice, is commensurate in scope with both inhibiting or reducing colonization by a bacterial pathogen and attenuating (a usage in the art to mean reduce or weaken) its virulence. Thus, the ability of c-di-GMP to inhibit colonization of *S. aureus* and infection in mice also means that it attenuated the virulence of *S. aureus* as well. Similarly, the other references listed in the attached Summary Table disclose a representative number of different gram-positive and gram-negative bacterial pathogens in which c-di-GMP or

another cyclic dinucleotide inhibited their ability to colonize and therefore reduced/attenuated their virulence.

The examiner has further asserted in the response to arguments that the specification does not give any working examples (i.e., in a challenged mice model) and thus fails to disclose any method for attenuating the virulence of any bacterial pathogen or for inhibiting or reducing colonization by any bacterial pathogen. The examiner further takes the position that the art has questioned the correlation between *in vitro* susceptibility and *in vivo* effectiveness.

Again, as argued previously and re-emphasized here, the examiner's contention that there is no working example provided in the specification, such as a challenged mouse model, is clearly incorrect. Example 8 on page 80 of the present specification (along with Fig. 15) provides just such a working example in which c-di-GMP inhibits colonization and infection (and thereby attenuates virulence) by *in vivo* challenge with *S. aureus* in a mouse mastitis model. Here, correlation between *in vitro* susceptibility and *in vivo* effectiveness is not necessary because applicant has directly demonstrated *in vivo* effectiveness. Such *in vivo* effectiveness against *S. aureus* is further supported by the experimental results discussed in the two declarations (filed and made of record on October 6, 2008 and September 15, 2009) and summarized in the attached Summary Table.

See references 2 to 6 in which the experimental results showing inhibition of colonization and reduced virulence were all obtained *in vivo*. Accordingly, the examiner's position regarding lack of working example and correlation between *in vitro* susceptibility and *in vivo* effectiveness is untenable.

The presently claimed invention is fully enabled by the guidance provided by the present specification combined with the high level of skill and knowledge of those in the art and mere routine experimentation.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 1-7, 9-21 and 28-30 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. Applicant believes that this rejection inadvertently includes claims 17-21 because claims 17-21 are specifically indicated by the examiner on the Office Action Summary page as being allowed. This rejection is respectfully traversed and argued below.

The examiner alleges that, absent a detailed and particular description of a representative number of the members of the genus of cyclic dinucleotides, the skilled artisan could not immediately recognize or distinguish members of the claimed genus of cyclic dinucleotides with the recited activities.

It should first be pointed out that the genus of cyclic dinucleotides is not large. The species of the genus all have two nucleotides with ribose sugar moieties cyclized in a head-to-tail arrangement. There are only a limited number of bases (abbreviated in the art as A, G, C, T, U, I) and therefore there are only a limited number of combinations of two bases in the cyclic dinucleotide. This is clearly orders of complexity/variability less than that of longer strings of nucleotides, whether cyclized or not. Referring to the chemical structure of c-di-GMP on page 9 of the specification and the 19 other examples of cyclic dinucleotides disclosed on pages 19-21, since the 3'-position on the ribose sugar moiety of a nucleotide is joined to the 5'-position of the partner ribose sugar moiety by a monophosphate (phosphodiester bond of nucleic acids) in a head-to-tail arrangement (no other arrangement is possible), then there is only a single 2'-position (besides the base at the 1'-position) on each ribose sugar moiety that can be varied. This 2' position can be a hydroxyl (e.g., ribonucleotide), a hydrogen (deoxy ribonucleotide) or a different substituent/moiety. Accordingly, the variability of cyclic dinucleotides is low, and the genus is on the order of hundreds of species at most. Applicant has described twenty different and specific species of this genus of cyclic dinucleotides in the present specification on pages 19-21. Such a number of specifically described species

in the specification is representative of a genus with species that number at most in the hundreds and would be sufficient for these of skill in the art to recognize that the applicant was in possession of the invention at the time the application was filed.

Furthermore, applicant has taught in the present specification that he expects cyclic dinucleotides, with c-di-GMP being the most preferred embodiment, to have the activity of attenuating virulence or inhibiting/reducing colonization of bacterial pathogens. It is only with regard to biofilm formation (and this is only with observations in two species, *Vibrio cholerae* and *Salmonella enteritidis*) that applicant discloses the possibility that c-di-GMP may enhance biofilm formation in some bacteria instead of inhibiting/reducing biofilm formation. However, applicant's teaching that, for biofilm formation, c-di-GMP and other cyclic dinucleotides can either enhance or inhibit biofilm formation, shows that the activity (enhancing or inhibiting biofilm formation) of c-di-GMP and cyclic dinucleotides was in applicant's possession. Determination of whether a cyclic dinucleotide enhances or inhibits biofilm formation is simply reduced to a quick and easy assay that can be performed in microtiter plates with a multitude of different cyclic dinucleotides to determine which specific cyclic dinucleotides will either enhance or inhibit biofilm formation as

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taught by the specification. Just because such a determination of biofilm inhibitor or enhanced may be needed does not affect the description of the invention to those of skill in the art.

Apart from biofilm formation, applicant has taught in the specification that he fully expects c-di-GMP and cyclic dinucleotides to attenuate virulence or to inhibit or reduce colonization. Accordingly, those of skill in the relevant art would certainly recognize that the claimed subject matter was indeed described in the specification (with a quick and easy assay to determine enhanced or reduced biofilm formation) in such a way as to convey possession of the presently claimed invention.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

In view of the above, the claims comply with 35 USC 112 and define patentable subject matter warranting their allowance. Favorable consideration and early allowance are earnestly urged.

Respectfully submitted,

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